

**DEPMEDS LABORATORY PROCEDURES  
DEPARTMENT OF CLINICAL SUPPORT SERVICES  
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL  
FORT SAM HOUSTON, TEXAS 78234-6137**

**MCCS-HCL**

**STANDING OPERATING PROCEDURE**

**01 September 02**

**ID-Micro Typing System – Gel Technique**

**1. Principle:**

ABO reverse (serum) grouping is designed to demonstrate the presence of the expected ABO blood group antibodies anti-A and/or anti-B by testing the serum or plasma with known A (A<sub>1</sub>) and B cells. Using a gel card, the presence/absence of anti-A and/or anti-B can be detected in the gel microtube. Agglutination indicates the presence of antigen/antibody reaction while lack of agglutination indicates the absence of antigen/antibody reaction.

**2. Specimen:**

- a. No special preparation of the patient is required prior to specimen collection.
- b. A completely clotted or EDTA or ACD anticoagulated sample drawn within three days of testing may be used.
- c. Fresh serum may be used to assure the presence of adequate complement and calcium. If plasma is used for the indirect antiglobulin test, complement-dependent antibodies may not be detected

**3. Equipment and Reagents**

a. Equipment

- 1) Test tubes (12 x 75 mm preferred for preparing cell suspensions)
- 2) Pipettes capable of delivering volumes of red cells 0.5mL and/or 1.0mL
- 3) Micropipettes capable of dispensing 10-12.5µL and/or 25µL and/or 50µL
- 4) ID-MTS Incubator □ (37± 2°C)
- 5) ID-MTS Centrifuge □ (895±25 RPMs)
- 6) ID-MTS Dispenser □ 0.5mL and/or 1.0mL
- 7) ID- MTS Work Rack □ or test tube and gel card racks

- 8) Thermometer capable of measuring  $37\pm 2^{\circ}\text{C}$
- 9) Serofuge capable of centrifuging 12 x 75 mm or other appropriate test tubes
- 10) Centrifuge capable of separating cells and serum/plasma
- 11) Screening cells, pooled cells, panels cells and/or reverse typing cells
- 12) ID-MTS Gel cards (IgG, Buffered Gel, A/B/D Reverse Grouping, etc.)
- 13) ID-MTS Diluent™ (1,2 or 2 Plus)

b. Reagents

- 1) MTS Diluent 1□, a bromelin enzyme solution
- 2) MTS Anti-A Card□, six microtubes of anti-A Gel
- 3) MTS Anti-B Card□, six microtubes of anti-B Gel
- 4) MTS Anti-A,B Card□, six microtubes of anti-A,B Gel
- 5) MTS Control Gel Card□, six microtubes of Control Gel
- 6) A<sub>1</sub> and B cells ( $3\pm 1\%$ )
- 7) MTS Diluent 2 Plus™, a hypotonic buffered saline solution containing EDTA
- 8) MTS Buffered Gel Card™, a buffered gel suspension

4. **Calibration:**

Not applicable. When the analyzer is turned on, it automatically performs a series of self-tests to verify hardware integrity. If an instrument Self-Test fails, an error message is displayed. Consult the Section 7 of the Operator's manual.

5. **Quality Control:**

**NOTE:** Prior to performing quality control, antibody detection and reverse grouping cells must be at a 0.8% cell concentration in the proper diluent. (Diluent 2 and Diluent 2 Plus respectively.) Dilute red blood cells for ABO and D QC (i.e., ORTHO Confidence Cells I and II) to 4% in MTS Diluent 1™.Daily

a. Test Procedure

1. Label the MTS Anti-IgG Card™ with the appropriate test information.

2. Remove the foil seal from the microtubes to be used for testing.
3. Using an appropriate pipette, add 50µL of each 0.8% reagent red cell sample to the labeled microtube.
4. Using an appropriate pipette, add 25µL of the QC antibody to the microtube(s).
5. Incubate at 37±2°C for 15 minutes. Refer to the package insert for comment on extending incubation times.
6. Centrifuge the gel card at the preset conditions of 895±25 RPMs for 10 minutes.
7. Read macroscopically the front and the back of each microtube and record reactions as described in the interpretation section of the corresponding MTS Gel Card package insert.

**a) ABO and D red cell QC-** Preparation of a 4% suspension of red cells, for A, B, and D red cell testing

**1) Method 1 (For 1-3 tests, from 3% cells)**

- a1. Label two test tubes for ORTHO Confidence Cells I and II, including lot number, date, time of preparation.
- b1. With an appropriate pipette, dispense 100µL of each red cell sample to the appropriately labeled tube and centrifuge for one (1) minute to pack the cells.
- c1. Decant to a dry-cell button (blotting is preferred) and add 50µL of MTS Diluent 1™.
- d1. Incubate the cell-diluent mixture for 10 to 20 minutes at room temperature.

NOTE: This formula was developed to better target the 4% cell suspension when working with small volumes.

**2) Method 2 (For up to 20 tests, from 3% cells)**

- a2. Label two test tubes for ORTHO Confidence Cells I and II, including lot number, date and time of preparation.
- b2. With an appropriate pipette, dispense 0.4mL of each red cell sample into an appropriately labeled tube. Centrifuge for one (1) minute to pack the cells.
- c2. Decant the supernatant and add 0.3mL of MTS Diluent 1™.
- d2. Incubate the cell-diluent mixture for 10 to 20 minutes at room temperature.

**c. Test Procedure**

1. Label two MTS A/B/D and Reverse Grouping Cards™, one for ORTHO Confidence Cell I and the other for Confidence Cell II.

2. Remove the foil seal from the microtubes.
3. Using an appropriate pipette, add 10-12.5µL of 4% ORTHO Confidence Cell I, diluted in Diluent 1™, to the Anti-A/-B/-D and Control microtubes of the labeled card. **Note: Cells may be added at any time after dilution as long as the centrifugation step begins within the 10 to 20 minute incubation time.**
4. Using an appropriate pipette, add 10-12.5µL of 4% ORTHO Confidence Cell II, diluted in Diluent 1™, to the Anti-A/-B/-D and Control microtubes of its labeled card. **Note: Cells may be added at any time after dilution as long as the centrifugation step begins within the 10 to 20 minute incubation time.**
5. See below for set-up of ABO reverse grouping QC, which may be performed in one of these two gel cards.
6. Centrifuge the gel cards at the preset conditions of 895±25 RPMs for 10 minutes.
7. Read the front and the back of each microtube macroscopically and record reactions as described in the interpretation section of the corresponding MTS Gel Card package insert.

### **ABO reverse grouping**

Preparation of a 0.8% suspension of reagent red cells in MTS Diluent 2 Plus™

Method 1 (For up to 60 tests; from a 3% cell suspension)

1. Label two test tubes A<sub>1</sub> and B; include lot number, date, and time of preparation.
2. With an appropriate pipette, dispense 1.0mL of each reagent red cell to the appropriately labeled tube. Centrifuge for one (1) minute to pack.
3. Decant the supernatant and add 3.0mL of MTS Diluent 2 Plus™ to each tube.
4. Mix gently. Final cell suspensions should be approximately 0.8% and are stable for 24 hours. For best results, suspensions should not be less than 0.6% or exceed 1.0%.

Method 2 (For up to 20 tests; prepared from packed cells)

1. Label two test tubes A<sub>1</sub> and B; include lot number and date and time of preparation. Prepare a volume of cells sufficient to provide 10µL of packed red blood cells of each reagent sample.
2. In separate labeled tubes, dispense 1.0mL of MTS Diluent 2 Plus™. Add 10µL of each of the packed reagent red blood cell samples to the labeled tubes.

- Mix gently. Final cell suspensions should be approximately 0.8% and are stable for 24 hours. For best results, suspensions should not be less than 0.6% or exceed 1.0%.

#### Test Procedure

- Label an MTS A/B/D and Reverse Grouping Card™. This testing may be performed in one of the gel cards used for red cell grouping QC as described above.
- Remove the foil seal from the microtubes.
- Using an appropriate pipette, add 50µL of 0.8% A<sub>1</sub> and B cells to the labeled Buffered Gel microtubes. Add 50µL of QC antibody to the Buffered Gel microtubes.
- Centrifuge the gel card at the preset conditions of 895±25 RPMs for 10 minutes.
- Read the front and the back of each microtube macroscopically and record reactions as described in the interpretation section of the corresponding MTS Gel Card package insert.

**RESULTS:** The following chart illustrates the expected results.

Component of QC System	Reagent Being Tested	Expected Test Result
ORTHO Confidence Antibody or other IgG-reactive blood group antibody/antibodies.	Screening cell(s)	1+ to 3+
ORTHO Confidence Antibody	A <sub>1</sub> reverse cell	1+ to 3+
	B reverse cell	1+ to 3+
Negative Control*	Screening cell(s)	0
ORTHO Confidence Cell I (AB rr)	Anti A	1+ to 4+
	Anti B	1+ to 4+
	Anti D	0
	Rh Control*	0

ORTHO Confidence Cell II (O R <sub>1r</sub> )	Anti A*	0
	Anti B*	0
	Anti D	1 to 4+
	Rh Control	0

## 10. References:

### REFERENCES:

Accreditation requirements manual. 6<sup>th</sup> ed. Bethesda, MD: American Association of Blood Banks, 1995:15-16.

Current package insert: ORTHO<sup>®</sup> Confidence System. Raritan, NJ: Ortho-Clinical Diagnostics Inc.

Standards for blood banks and transfusion services. 18<sup>th</sup> ed. Bethesda, MD: American Association of Blood Banks, 1997.

Current package insert: Anti-Human Globulin Anti-IgG (Rabbit) MTS Anti-IgG Card. Pompano Beach, FL: Micro Typing Systems, Inc.

Current package insert: A/B/D and Reverse Grouping Card<sup>™</sup>. Pompano Beach, FL: Micro Typing Systems, Inc.

Current package insert: MTS Diluent 1<sup>™</sup> Red Blood Cell Diluent. Pompano Beach, FL: Micro Typing Systems, Inc.

Current package insert: MTS Diluent 2<sup>™</sup> Red Blood Cell Diluent. Pompano Beach, FL: Micro Typing Systems, Inc.

Current package insert: MTS Diluent 2 Plus<sup>™</sup> Red Blood Cell Diluent. Pompano Beach, FL: Micro Typing Systems, Inc.

Current package insert: Reagent Red Blood Cells Selectogen<sup>™</sup>. Raritan, NJ: Ortho-Clinical Diagnostics Inc.

Current package insert: Reagent Red Blood Cells Surgiscreen<sup>™</sup>. Raritan, NJ: Ortho-Clinical Diagnostics Inc.

Current package insert: Reagent Red Blood Cells ORTHO<sup>®</sup> Pooled Screening Cells. Raritan, NJ: Ortho-Clinical Diagnostics Inc.

Current package insert: Reagent Red Blood Cells AFFIRMAGEN<sup>™</sup>. Raritan, NJ: Ortho-Clinical Diagnostics Inc.

